



NASA ASTROBIOLOGY INSTITUTE ANNUAL REPORT YEAR [July 2003 - June 2004]

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Annual Reports :: Year 6 :: Virus

Focus Groups: Virus

Chairs:

Kenneth Stedman , Baruch Blumberg

Project Progress

I. **Brief background and proposed future activities:**

1. **Background** : The purpose of the NAI Virus Focus Group, founded in September 2002, is to study the role of viruses in astrobiology. It addresses the question of how these infectious agents, that are extremely widely distributed and abundant on and in contemporary Earth, were involved in the origin and early periods of life on Earth and elsewhere.
2. A workshop/field trip is planned to take place from June 22 nd to 24 th at Mono Lake and the Long Valley Caldera in the Mammoth Lake region in eastern CA. A full report will be submitted in our next annual report. It will be a collaborative research project including Focus Group members (and others) who require material collected on the field trip for their research. We will also invite prominent virologists and microbiologists not currently directly involved in astrobiology to take part in the project in order to introduce them to the excitement of the program. Participants will have different observational and experimental methods and different hypotheses to test. Since many of the observations will be made on specimens collected in the same place, and on some of the same specimens it will be possible for those who wish to do so to combine their data for more complex meta–analyses. The collected samples will be archived for future use.
3. An informal meeting will be held at the American Society for Virology annual meeting in Montreal , Canada (August 2004).

II. Focus Group Activities

1. A Focus Group meeting and Workshop was held at Portland State University . In Portland , OR, October 16–17, 2003 (see below).
2. A second Focus Group Meeting was held at the Astrobiology Science Conference at NASA Ames Research Center (April 2004).
3. A highly successful symposium on viruses and astrobiology organized by members of the Virus Focus Group and others was on the program of the Third Astrobiology Science Conference (March 28 th – April 1 st , 2004). The program for this symposium is attached.

4. We propose publishing the proceedings of the Virus Focus Group Workshop held in Portland, OR, October 16–17, 2003 (see below), and the field trip/workshop at Mammoth Lakes in June, 2003, in the Journal “Astrobiology”. The Editor, Dr. Cady, has welcomed the submission of these papers. We expect to submit these by September 1, 2004, at the latest. Drafts of four reports have already been received.

III. **Relevance to the Astrobiology Roadmap** listed in the order given in the Astrobiology Roadmap brochure.

1. **Objective 2.1 Mars exploration.** If signatures of the presence of virus can be determined it would be appropriate to search for these on Mars, or in returned material from Mars. Virus fossils have not been reported, but if they can be identified they would also be the subject for searches. Members of the focus group are investigating virus mineralization in the laboratory as evidence for the potential existence of these fossils.
2. **Objective 4.1 Earth's early biosphere.** Investigations similar to those described for Mars would be appropriate.
3. **Objective 4.2 Foundations of complex life.** Viruses may have appeared on Earth soon after the development of cells, or conceivably before the event if earlier forms of viruses could exist independent of cells. Evidence was presented at the recent workshop for virus types that may have pre-dated the split of the Eukarya, Archaea, and Bacteria lineages.
4. **Objective 4.3 Effects of extraterrestrial events upon the biosphere.** The effect of impacts on microorganisms, including viruses, is a major theme for this objective. Virus persistence and stability are being tested by focus group members.
5. **Objective 5.1 Environment-dependent, molecular evolution in microorganisms.** Viruses would be included in the microorganisms studied, including bacteriophage, i.e., viruses of microorganisms. One research group in the focus group has this as the main focus of their research.
6. **Objective 5.2 Coevolution of microbial communities.** The role of viruses in microbial mats in different environments, particularly in lateral gene transfer.
7. **Objective 5.3 Biochemical adaptation to extreme environments.** The study of viruses from extreme environments under extreme conditions and, in particular, their ability to survive for very long periods of time, is the focus of two research groups in the virus focus group.
8. **Objective 6.1 Environmental changes and the cycling of elements by the biota, communities, and ecosystems.** Viruses will be included among the life forms to be studied under these conditions of change. (See Objective 5.1).
9. **Objective 6.2 Adaptation and evolution of life beyond Earth.** Viruses are important life and life-accompanying forms that should be studied beyond their planet of origin. The effect of the space environment on acute and chronic viral infection of humans and other animals and plants is an important part of the humans in space program.

IV. *Relevance to space missions and astrobiology technology development.* Decisions on future space missions will depend on future development of the field of viral astrobiology. This includes missions to Mars, Europa, and the other moons of Jupiter. The study of viruses in the International Space Station (ISS) and on free flyers and the exposure of viruses to the space environment are additional programs. Technology will be required for the robotic collection of viral specimens in extreme environments on Earth, under the sea, and on other planets and moons. Technology for the *in situ* detection and identification of viruses will be required. This will also have significant medical applications. The study of viruses of extremophiles in animal and plant pathology will require new technology.

V. *Why continuation of the Virus Focus Group would be valuable.* The field of viral astrobiology is in its infancy. Very little is known about the epidemiology of viruses in extreme environments, nor about their molecular biology, replication strategies, gene expression, and other characteristics. There have been few investigations of viruses in the space environment and their possible role in pathology in spacefarers. We have outlined above how virus studies fit within the Roadmap objectives; very few studies in this area have been accomplished. We envision the Virus Focus Group as a mechanism to focus attention on these issues and to conduct field trips and collaborative studies. In the future we intend to apply for research grants from the National Institutes of Health (NIH) and other agencies to fund virus research stimulated by the astrobiology endeavor.

VI. *Final Report for the first year.* The major activity for the first year has been a Virus Focus Group Workshop. The results are summarized below:

**Report on the
NASA ASTROBIOLOGY INSTITUTE VIRUS FOCUS GROUP WORKSHOP
The Simon Benson House, Portland State University , Portland OR
October 16–17, 2003**

Ken Stedman, and Baruch Blumberg convened the first meeting of the NASA Astrobiology Institute (NAI) Virus Focus Group at Portland State University , October 16–17, 2003 . An additional organizer of the meeting was David Smith, of the NAI Team at the University of Rhode Island , who was unable to attend.

Ken Stedman welcomed the participants. The program of the meeting, a list of the attendees, and a list of the members of the Focus Group is appended.

Baruch Blumberg described the origin of the NAI Focus Groups; they were initiated at a meeting of the NAI Executive Council at NASA Ames Research Center on 12.18.99. The nature of the NAI Focus Groups were not defined at that time with the expectation that each Focus Group would determine its own character. The NAI Virus Focus Group was approved on 09.09.02 at the Executive Council meeting at Pennsylvania State University .

There are several interesting questions related to viruses and astrobiology. Were viruses an early form of life on Earth? Could they have preceded the origin of cells and survived, flourished and replicated cell-free in an earlier form? Did viruses exist on early Mars, or elsewhere away from Earth? If so,

how could their presence be detected? Is it possible to identify fossil viruses in the paleontological record on Earth or other places? Are there geological and/or geochemical signatures of viruses (that can exist in vast quantities in even the most hostile of environments) that could be detected with appropriate instruments? Viruses live in cells and many integrate into the genome of their hosts. These could be transmitted to subsequent generation in a manner similar to a mutation. Can viruses act as important agents of evolution? If so, is this an ongoing program? If an RNA world preceded the DNA world, were viruses involved in this development?

A particularly interesting question relates to the survivability of viruses in the space environment, a matter of interest to astrobiology and fundamental space biology. Can viruses and their host cells and organism survive in some form in the space environment and, in particular, in the interior of objects such as meteorites. What is the effect of the space environment on space farers chronically infected with a virus? Will viruses that are normally not pathogenic become so after long periods of time in space? These are issues of basic scientific interest that relate to the problems of human space flight.

There has been a major interest in astrobiology on the study of extremophiles, lifeforms that could have arisen and prevailed under the demanding conditions of early earth and other objects in the cosmos. The viruses of microorganisms – phages – often provide genes that are important to the life cycle of their bacterial hosts. For example, a phage is responsible for the toxins of the cholera vibrio. How common are phages in extremophiles and what are their distribution, diversity and other characteristics? Research on this topic was a major part of the Focus Group workshop.

Many of these questions were considered in the workshop, either directly or indirectly, and the details of the presented papers will be given in the final report. (**Sherry Cady** the editor of the journal “Astrobiology,” who attended the workshop and is a member of the Virus Focus Group and the NASA–Ames NAI, has agreed to consider the final report for publication.) A brief summary of the presentations will be given here including information on field sites used by the investigators. (See attached program.)

Luis Ruedas discussed the ecology and medical virology of the Hantaan viruses and the effects on pathogenesis of the interaction of the host, the virus, and the environment. There are very different clinical manifestations depending on the local ecology. In some environments and geographic locations the disease can be severe and in others there are little or no clinical disadvantages. Host polymorphic susceptibility genes influence many chronic infections; beneficial effects to the host might be expected in some circumstances. Ruedas and his colleagues examined the possible beneficial effects of long–term infection with the virus on maximal aerobic capacity in infected experimental mice and longevity in their natural environment. They also determined that infection rates for Hanta viruses in humans were decreased in ecologies where there is greater diversity in the putative mammalian definitive hosts (i.e., mice and other rodentia). Fieldwork for this project included the Four Corners region of the southwest USA , the location for recent epidemic of Hantaan Virus infection, and other sites, including

Panama and greenspaces in Portland , OR .

Paul Turner studies the dynamics of evolution in bacteria, viruses and other microorganisms. He is now directing his attention to the role viruses and other gene transfer agents on the dynamics of evolution, particularly the role of genetic reassortment or “sex” in viral fitness. He and his colleagues are utilizing a field site on St. Lucia in the Windward Islands of the Caribbean Sea where there are several geothermal springs located near each other in a volcanic caldera. They provide an opportunity for field observations on differences in evolutionary directions in similar, but not identical, sites under different selective pressures.

Ken Stedman has done intensive studies on the viruses of the extremely thermophilic archaeon *Sulfolobus*. Many species have been isolated and sequenced, and their molecular biology, including means of replication, has been determined. They have unusual, one could even say bizarre, physical morphologies that have not been previously described. Stedman and his colleagues have published (Prangishvili *et al.*, *Trends in Microbiology* , 9,39, 2001) a fascinating observation, echoed in other presentations in this Workshop: “... few ORFs in the genomes of these viruses show any similarity to ORFs encoding proteins in other viruses or organisms.” Stedman's research has produced a major body of data on life forms of particular interest to the Virus Focus Group. Collections of *Sulfolobus* species and their viruses have been made in thermal sites in Japan , Iceland , North America , Kamchatka , and elsewhere. Stedman showed images of the Kamchatka field site, a location with 43 active volcanoes and many extraordinary geothermal locations. He also discussed new results indicating that some virus morphotypes may have predated the Eukarya–Bacteria–Archaea divergence.

Ray Kepner has studied microbial mats in extreme locations with an emphasis on their “physiology” and ecological relations. He described mats that grow on the bottom of Antarctic ice covered lakes. Some rise in pinnacles, detach, float to the underside of the icy covering, migrate through the ice (a process that may take as much as 11 years), and rise to the surface where they dehydrate and blow off in the heavy winds of the region to become part of the surrounding landscape. This is an interesting example of the delivery of carbon–containing material from a marine location to land. There was a discussion of the role of the “quorum” sensing molecules that are important in the medical treatment of biofilms that form on implanted prosthesis such as artificial or exobiotic heart valves. Kepner suggested collaborations with others in the group interested in the identification and characterization of viruses in the material he is collecting.

Forest Rohwer presented a series of studies on the sequencing of viruses, mostly phages, from marine environments in San Diego and La Jolla , CA . He compared these to the sequences obtained from human feces. There is a remarkable diversity in the viral and phage types with striking differences between the two marine locations. He has made estimates of the prevalence of life forms in seawater. There are 10×10^6 viruses and phages per milliliter of seawater compared to 1×10^6 bacteria and lesser amounts of other forms (for example, algae). The oceans appear to be the biggest pool of biological material on earth with viruses being the most common form. His research

demonstrated that the genomic analysis of uncultured marine viral communities is feasible, pointing toward the possibility of similar studies elsewhere. Other collection sites included geothermal locations in Mammoth and Mono Lakes in eastern CA and the geothermal energy sites at El Centro and Imperial Spa, in southern CA. Twenty percent of the phage genomes did not have any close homologs in the sequence databases, a report similar to that of Stedman's (above).

This observation requires further support. It would be fascinating if phages, and particularly phages of extremophilic Bacteria and Archaea have unique genomic sequences. This could generate the hypothesis that they may have had a separate origin on Earth or that they originated elsewhere. These speculative hypotheses seem unlikely, but would certainly be interesting to test.

Alice Ortmann presented the paper from Curtis Suttle's group at the University of British Columbia, as Azeem Ahmed was unable to attend. Their laboratory has studied the viruses of the algae *Heterosigma akashiwo* that affects farmed fish. The collections were accomplished from a Canadian Coast Guard vessel in the Straits of Georgia (east of Vancouver Island, BC). Collections were also made in the submarine hydrothermal vent field on Endeavour Ridge, BC. These collections add to the growing database on viruses of extremophiles. They also observe large amounts of diversity in their bacteriophage samples from all samples both spatially and temporally distributed. The group has been one of the leaders in the discovery and analysis of bacteriophage and viruses in marine environments.

Grieg Steward, the University of Hawaii, presented a paper that was not on the original program. He reported on an extensive series of studies of virally infected cells in widespread marine locations. These included, among others, sites in Long Island Sound (NY), in the eastern and western Caribbean, in the Gulf Stream and the Sargasso Sea, Mono Lake, CA, and under the arctic ice. The latter collections were made by Grieg on board a nuclear submarine during an extended undersea and under ice voyage from Groton, CN to Seattle, WA that included a single surfacing at the North Pole. This was part of the SCICEX 97 research program. Some of his studies were multiple collections over time, for example; HOTS, Hawaiian Ocean Time Series, and BAT, Bermuda Atlantic Time series. The extensive data set included information on virus infectivity, mortality, diversity, etc.

CONCLUSIONS AND PLANS FOR FUTURE MEETINGS AND ACTIVITIES.

The study of the viruses of extreme locations is currently, in large part, in a data collecting and inductive phase. That is, the data is being collected on which the hypotheses that will move the field forward will be based. The papers presented, which represent only a fraction of ongoing research, indicate how rich this field is and will become. It is already clear that there are many viruses and phages in extreme environments, that there is great diversity, and that the viruses are very unusual in their appearance and molecular biology. The phages and the microorganisms they infect produce a wide range of unusual proteins and enzymes that will have valuable, if unpredictable, applications in research, medicine, and industry.

The NAI Virus Focus Group has the potential of becoming an active and effective group of scientists who can help develop this important program directed towards understanding the origins, evolution, distribution and future of life on Earth and in the Universe.

***NASA Astrobiology Institute Virus Focus Group Workshop: October
16–17, 2003
PROGRAM***

The Simon Benson House, Portland State University , Portland Oregon .

Thursday, October 16 th , 2003

Session I. The Origin and Evolution of Viruses

9:00 – 9:15 Welcome and Introductions *Ken Stedman, Portland State University*

Baruch Blumberg, NAI/Fox Chase Cancer Center

9:15 – 9:45 The indissoluble bond: Virus–host interactions in an ecological context:

Luis Ruedas, Portland State University

9:45 – 10:45 Experimental evolution in viruses: *Paul Turner, Yale University*

10:45 – 11:15 Break (Visit to PSU Electron Microscopy Facility)

11:15 – 12:00 Discussion – *Participants*

12:00–13:30 Lunch

Session II. Viruses in Extreme Terrestrial Environments

13:30 – 14:30 Hot Viruses *Ken Stedman, Portland State University*

14:30 – 15:00 Break (Visit Stedman Lab, Center for Life in Extreme Environments, PSU)

15:00 – 15:30 Lysogenic cyanophage from polar microbial mats: Neglected, selected, injected, infected, inspected and... detected? *Ray Kepner, Marist College*

15:30 – 17:00 Discussion – *Participants*

Friday, October 17 th , 2003

Session III. On the Ubiquity of Viruses

9:00 – 10:00 Viral Biodiversity *Forest Rohwer, San Diego State University*

10:00 – 10:30 Break

10:30 – 11:00 Virus Diversity in Marine Environments *Alice Ortmann, U. British Columbia*

11:00 – 11:30 Marine Viral Ecology *Grieg Steward, U. Hawaii*

11:00 – 12:00 Discussion – Participants

12:00 – 13:30 Lunch

Session IV. Future Directions of the NAI Virus Focus Group

13:30 – 15:00 Discussion Leader *Baruch Blumberg* . Potential Agenda Items:
Next Meeting? Election/Nomination of Chair(s).

Workshop Participants (with e-mail addresses)

Baruch Blumberg, Fox Chase Cancer Center/NAI: Baruch.Blumberg@fccc.edu
Gautam Dutta, Evergreen State College: dutgau09@evergreen.edu
Todd Gary, Tennessee State University: tgary@coe.tsuniv.edu
Grieg Steward, University of Hawaii : grieg@hawaii.edu
Emma Hambley, University of British Columbia : ehambly@eos.ubc.ca
Ray Kepner, Marist College : Raymond.Kepner@marist.edu
Brig Klyce, Astrobiology Research Trust: bklyce@panspermia.org
Bette McKnight, North Carolina A&T: mcknight@ncat.edu
Alice Ortmann, University of British Columbia : ortmann@interchange.ubc.ca
Raul Raya, Evergreen State College: rayar@evergreen.edu
Forest Rohwer, San Diego State University : forest@sunstroke.sdsu.edu
Anne Rosenthal, Freelance Science Writer: ANNEMROSENTHAL@cs.com
Paul Turner, Yale University : paul.turner@yale.edu
Jay Withgott, Freelance science writer: jwithgott@msn.com
David Boone, Portland State University : dboone@pdx.edu
Sherry Cady, Portland State University : cadys@pdx.edu
Anna-Louise Reysenbach, Portland State University : areysenbach@pdx.edu
Luis Ruedas, Portland State University : ruedas@pdx.edu
Kenneth Stedman, Portland State University : kstedman@pdx.edu

Astrovirology Session, Absscicon, 2004. March 31 st 2004 .

	<i>Astrovirology – Ken Stedman and Baruch Blumberg, Chairs</i>		
2:00	Roger Hendrix	University of Pittsburgh	Bacteriophage genomics: evolution of the majority

2:30	Stephen S. Morse	Columbia University	The Origins and Evolution of Eukaryotic Viruses: Are Viruses Inevitable?
3:00	Mark J. Young, Kenneth Stedman, Blake Wiedenheft, Jamie Snyder, George Rice, Josh Spuhler, Francisco Roberto, Liang Tang, Trevor Douglas and J. E. Johnson	Montana State University	Viruses from Yellowstone's high temperature acidic environments.
3:15	Kenneth Stedman, Yannick Combet-Blanc, Blake Wiedenheft, Adam Clore, George Rice, Jamie Snyder, Josh Spuhler, Luisa Zoeller, Francisco Roberto, Mark J. Young, Wolfram Zillig	Portland State University	Genetics and Genomics of Fuselloviruses of hyperthermophilic Archaea
3:30	Paul Turner	Yale University	Why is experimental evolution important for astrobiology?
3:45	Baruch Blumberg	Fox Chase Cancer Center	Astrovirology. The NAI Focus Group.

Current members of NAI–Virus Focus Group (e–mail list): NAI members in **bold** :

First	Last	E–mail Address
Steve	Abedon	microdude+@osu.edu
Azeem	Ahmed	aahmad@eos.ubc.ca
John	Baross	jbaross@u.washington.edu
Baruch	Blumberg	Baruch.Blumberg@fccc.edu
David	Boone	booned@pdx.edu
Andy	Brabban	brabbana@evergreen.edu
Sherry	Cady	cadys@pdx.edu
Simon	Clemett	simon.j.clemett1@jsc.nasa.gov
Rita	Colwell	rcolwell@nsf.gov

Jody	Deming	<i>jdeming@u.washington.edu</i>
Steve	D'Hondt	<i>dhondt@gso.uri.edu</i>
Gautam	Dutta	dutgau09@evergreen.edu
Todd	Gary	<i>tgary@coe.tsuniv.edu</i>
Grieg	Steward	grieg@hawaii.edu
Rose	Grymes	<i>Rosalind.A.Grymes@nasa.gov</i>
Emma	Hambly	ehambly@eos.ubc.ca
Fairhead	Heather	hf@phicotherapeutics.co.uk
Roger	Hendrix	rhx@pitt.edu
Rask	Jon	<i>jrask@arc.nasa.gov</i>
Ray	Kepner	Raymond.Kepner@marist.edu
Brig	Klyce	bklyce@panspermia.org
Betty	Kutter	kutterb@evergreen.edu
Vansessa	Lancaster	<i>vanessa.lancaster@asu.edu</i>
Teresa	Longanzo	teresa.g.longanzo@jsc.nasa.gov
S.V.	Manjunath	svmanju@indiatimes.com
David	McKay	<i>David.s.mckay@jsc.nasa.gov</i>
Bette	McKnight	<i>mcknight@ncat.edu</i>
Alice	Ortmann	ortmann@interchange.ubc.ca
John	Paul	jpaul@seas.marine.usf.edu
Raul	Raya	rayar@evergreen.edu
Anna-Louise	Reysenbach	areysenbach@pdx.edu
Forest	Rohwer	forest@sunstroke.sdsu.edu
Anne	Rosenthal	ANNEMROSENTHAL@cs.com
Lynn	Rothschild	<i>Lynn.J.Rothschild@nasa.gov</i>
Luis	Ruedas	ruedas@pdx.edu
Andrew	Schuerger	schueac@kscems.ksc.nasa.gov
Robert	Siegel	siegelr@stanford.edu
David	Smith	<i>dcsmith@gso.uri.edu</i>
Ken	Stedman	kstedman@pdx.edu
Andrew	Steele	<i>a.steele@gl.ciw.edu</i>
Matt	Sullivan	mbsulli@MIT.EDU
Curtis	Suttle	csuttle@eos.ubc.ca
Paul	Turner	paul.turner@yale.edu
Kokjohn	Tyler	tkokjo@midwestern.edu
James	Van Etten	jvanetten@unlnotes.unl.edu
Llyd	Wells	<i>chimera1@ocean.washington.edu</i>
Michael	Wendorf	Mike.Wendorf@kp.org
Frances	Westall	<i>westall@cnrs-orleans.fr</i>
Jay	Withgott	jwithgott@msn.com
Mark	Young	myoung@montana.edu

Roadmap Objectives

- **Objective No. 2.1:** Mars exploration
- **Objective No. 4.1:** Earth's early biosphere

- **Objective No. 4.2:** Foundations of complex life
- **Objective No. 4.3:** Effects of extraterrestrial events upon the biosphere
- **Objective No. 5.1:** Environment–dependent, molecular evolution in microorganisms
- **Objective No. 5.2:** Co–evolution of microbial communities
- **Objective No. 5.3:** Biochemical adaptation to extreme environments
- **Objective No. 6.1:** Environmental changes and the cycling of elements by the biota, communities, and ecosystems
- **Objective No. 6.2:** Adaptation and evolution of life beyond Earth

Mission Involvement

<i>Mission Class*</i>	<i>Mission Name (for class 1 or 2) OR Concept (for class 3)</i>	<i>Type of Involvement**</i>
3		Background
		Research

* Mission Class: Select 1 of 3 Mission Class types below to classify your project:

1. Now flying OR Funded & in development (e.g., Mars Odyssey, MER 2003, Kepler)
2. Named mission under study / in development, but not yet funded (e.g., TPF, Mars Lander 2009)
3. Long–lead future mission / societal issues (e.g., far–future Mars or Europa, biomarkers, life definition)

** Type of Involvement = Role / Relationship with Mission

Specify one (or more) of the following: PI, Co–I, Science Team member, planning support, data analysis, background research, instrument/payload development, research or analysis techniques, other (specify).

Decisions on future space missions will depend on future development of the field of viral astrobiology. This includes missions to Mars, Europa, and the other moons of Jupiter. The study of viruses in the ISS and on free flyers and the exposure of viruses to the space environment are additional programs. Technology will be required for the robotic collection of viral specimens in extreme environments on Earth, under the sea, and on other planets and moons. Technology for the in situ detection and identification of viruses will be required. This will also have significant medical applications. The study of viruses of extremophiles in animal and plant pathology will require new technology.

Field Expeditions

Field Trip Name: Mono/Mammoth Workshop

Start Date: 22 June 2004

End Date: 24 June 2004

Continent: North America

Country: USA

State/Province: California

Nearest City/Town: Mammoth Lakes

Latitude: Multiple

Longitude: Multiple

Name of site(cave, mine, e.g.): Hot Springs

Keywords: Viruses

Description of Work: Water samples were collected for virus and host analysis at high temperature (>70C) locations on Paoha island in Mono lake and hot springs at Hot Creek and Little Hot Creek in the Long valley caldera near Mammoth Lakes.

Members Involved: